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Issued June 10, 1915.

Hawaii Agricultural Experiment Station
HONOLULU

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PRESS BULLETIN No. 50*

The Effect of Arsenite of Soda on the Soil

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Introduction

The use of poisonous chemicals as a means of destroying weeds, while not a new idea, until comparatively recent years found little application in Hawaiian agriculture. About five years ago this Station carried on experiments[1] with various chemicals, the results of which indicated sodium arsenite to be a very effective and economical weed destroyer.

At the present time this means of controlling weed growth has long since passed the experimental stage and is now quite widely applied. Especially is it applicable in the rainy sections where the growth of weeds is profuse and cultivation is often impossible without puddling the soil.

Considerable credit is due Mr. W. A. Anderson, Manager of the Nahiku Rubber Co. for the first successful application of this

*Note.—This Bulletin, as well as Press Bulletin No. 48, entitled "Suppression of Weeds Among Pineapples by Arsenite of Soda Spray," and Press Bulletin No. 49, entitled "A Cheap and Effective Home-made Plank Drag," was prepared under the direction of Dr. E. V. Wilcox, Special Agent in Charge, previous to Jan. 1, 1915.

chemical on a large scale in these islands. On large areas on the rubber plantations plowing has been entirely replaced by the arsenical spray in the last five years. A further extensive test has been made by the Parker Ranch as a means of eradicating such weeds as thimble berry and German ivy from their pasture lands. Since these successful applications the use of this spray has increased until it has entirely replaced hoeing on some plantations and partially replaced plowing, even in the dry sections.

Object of the Work

The status of arsenite of soda as a weed destroyer is firmly fixed, from both a practical and an economical standpoint. But there still remains to be considered the effect of this chemical upon the soil and the growth of plants. Comparatively little work of this nature has been done but it is quite generally conceded that plant growth is materially affected by the presence in the soil of this chemical. The Colorado Experiment Station[2] has made some investigations upon the death of fruit trees excessively sprayed with insoluble arsenical compounds, and the Bureau of Chemistry of the U. S. Department of Agriculture[3] has investigated the death of plants as a result of arsenic settling from smelter fumes. In all cases arsenic has been found in both the soil and the plants in appreciable amounts.

In view of the above facts and the increase in use of arsenite of soda in the Hawaiian islands, the Station undertook an exhaustive study of the effect of this chemical upon plant growth and its physical, chemical and biological activities in the soil. While no deleterious effects have appeared through its use thus far, yet it is highly essential that we should be able to recognize them immediately should they appear.

Preparation of the Spray.

Several formulas have been recommended for the preparation of this spray. They all involve the boiling of white arsenic with sal soda or caustic soda, the two being added in sufficient quantities to combine, forming soluble sodium arsenite. When thus boiled there are two salts of sodium arsenite possible of formation the acid salt ($Na_2O.\ 2\ As_2O_3\ 2\ H_2O$) and the neutral salt ($Na_2O.\ As_2O_3$). Therefore the resulting compound depends upon the proportion of the two ingredients. The following equations illustrate:

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(1) $Na_2CO_3 .10H_2O + As_2O_3 = Na_2O. As_2O_3 + CO_2 + 10H_2O$
(2) $2NaOH + As_2O_3 = Na_2O. As_2O_3 + H_2O$
(3) $Na_2CO_3 .10H_2O + 2As_2O_3 = Na_2O .2As_2O_3 + CO_2 + 10H_2O$
(4) $2NaOH + 2As_2O_3 = Na_2O .2As_2O_3 + H_2O$

Equations 1 and 2 represent proportions for the formation of the neutral salt. By calculating from these two relationships it appears that it will require approximately 1.5 pounds sal soda or only 0.4 of a pound of caustic soda to react with 1 pound of white arsenic to form neutral sodium arsenite. Equations 3 and 4 represent the proportions at which the two react to form the acid salt. From these it can be shown that approximately 1 pound of sal soda combines with 1½ pounds of arsenic, and 1 pound of caustic soda combines with 5 pounds of arsenic to form the acid salt.

The effectiveness of the two salts as weed destroyers is very much the same. However, from an economical standpoint the neutral salt which has given excellent results upon most weeds is as follows[4]:

2 pounds sal soda
1 pound arsenic
1 gallon water.

Boil this mixture until clear, then dilute to about 24 gallons for spraying. Instead of 2 pounds sal soda, 7 ounces of caustic soda will produce the same results. However, the use of the latter salt involves a certain degree of danger to the laborers who may be required to handle it while the sal soda is practically harmless.

On Olaa plantation where the use of poisonous sprays has been given a thorough test the acid sodium arsenite is in use. The following formula has been adopted and is very effective:

20 pounds arsenic
4 to 5 pounds caustic soda
10 gallons water.

This mixture is boiled for 10 to 15 minutes and the whole diluted to 400 gallons. Instead of 4 pounds caustic soda approximately 13 pounds sal soda would be required.

Effect on plant growth.

In order to gain some information with reference to the effect of arsenite of soda upon plant growth a series of pot experiments

was carried out. For these experiments three soils were used and three crops. The soils included the heavy red clay type No. 1; a brown clay type No. 2; and a highly organic silt, No. 3. The soils were also of different chemical types and partial analyses are herewith inserted for reference:

ANALYSES OF SOILS USED.

Soil	Insol. matter per cent	Iron Oxide per cent	Alum. Oxide per cent	Lime per cent	Magnes. per cent	Potash per cent	Phos. Acid per cent	Vol. and Organic Matter per cent	Moisture per cent
1	33.17	21.08	17.70	.88	1.01	.16	.68	12.65	8.67
2	35.37	35.40*		.82	6.24	1.34	.35	11.55	7.36
3	32.69	10.13	12.59	2.63	1.09	.14	1.02	13.08	25.46

The crops used were millet, cowpeas and buckwheat, all of which have been found to give excellent growth in pots. Neutral sodium arsenite was applied to the pots in varying amounts as shown in the following table:

EFFECT OF ARSENIC ON PLANT GROWTH.

BUCKWHEAT

Pot	Arsenic in soil per cent.	Wt. of air dry plants gms.	No. of plants	Wt. of air dry plants gms.	No. of plants	Wt. of air dry plants gms.	No. of plants
1	.011	15.0	5	16.0	5	44.6	5
2	.005	7.2	5	16.4	5	45.5	5
3	.010	12.8	5	18.7	5	51.0	5
4	.015	8.3	4	15.0	5	49.3	5
5	.050	..	..	8.5*	3	14.8	3
6	.100	..	..	11.6*	5	..	..
7	.250	..	..	..	..	..	..
8	check	22.0	5	13.8	5	45.3	5

COWPEAS.

Pot	Arsenic in soil per cent.	Wt. of air dry plants gms.	No. of plants	Wt. of air dry plants gms.	No. of plants	Wt. of air dry plants gms.	No. of plants
1	.001	82.5	4	161.0	4	206.5	5
2	.005	65.0	4	61.0	2	107.0	2
3	.010	40.0	4	92.0	1	..	..
4	.015	49.0	4	114.0*	5	22.0	1
5	.050	12.2*	4	39.0*	2	14.0*	1
6	.100	..	..	..	..	..	..
7	.250	..	..	..	..	..	..
8	check	51.5	4	129.5	3	202.0	5

MILLET

Pot	Arsenic in soil per cent.	Wt. of air dry plants gms.	No. of plants	Wt. of air dry plants gms.	No. of plants	Wt. of air dry plants gms.	No. of plants
1	.001	14.5	5	23.5	5	73.0	5
2	.005	8.2	5	20.5	5	26.5	5
3	.010	6.0	5	19.5	5	69.5	5
4	.015	7.2	5	31.7	5	68.0	5
5	.050	..	..	16.5*	5	20.5*	5
6	.100	..	..	..	..	..	..
7	.250	..	..	..	..	..	..
8	check	10.0	5	25.0	4	64.2	4

*Represents weight of second planting, the first having died.

In some of the pots the seed germinated but the plants soon turned brown and died. All pots in which plants died at first planting were replanted and in several instances the seeds ger-

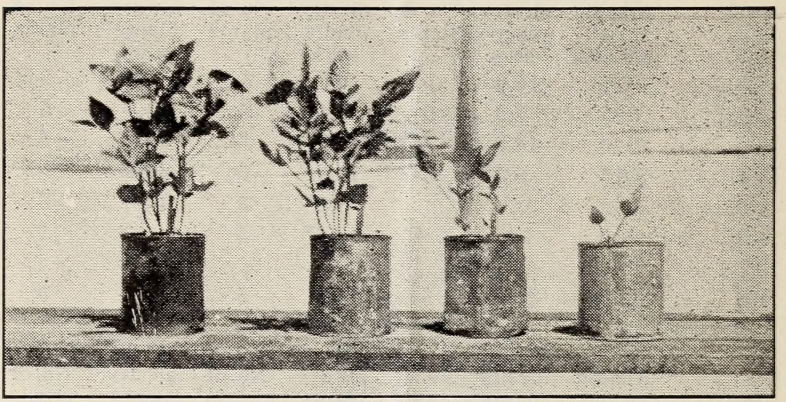

Fig. 1—Cowpeas—Soil No. 3
From left to right, Pots No. 8, 1, 2, 4.

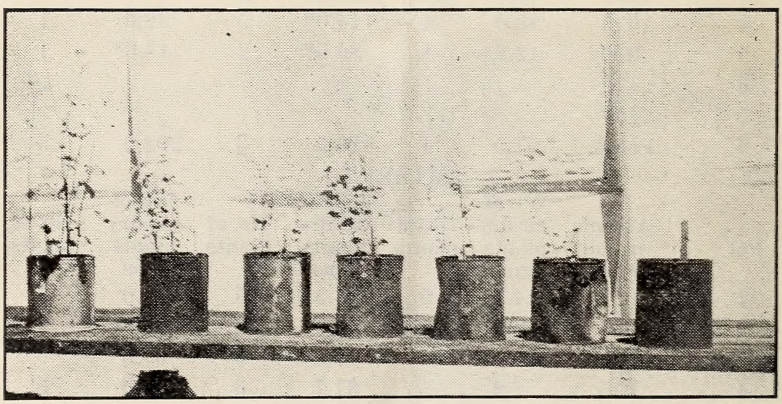

Fig. 2—Buckwheat—Soil No. 1
From left to right, Pots No. 8, 1, 2, 3, 4, 5.

minated at second planting and though much stunted in growth reached maturity.

These plants indicate strongly that the effect of sodium arsen-

ite on plant growth depends upon the resisting power of the plant and upon the chemical and physical nature of the soil. It might be said that in small quantities the plant growth is stimulated in most instances, but when added at the rate of .10 to .25 per cent, plant growth is practically impossible. No fertilizers were added to these pots and it is highly probable that their addition might influence the action of he arsenic.

The accompanying plate (Figs. 1, 2 and 3) illustrates more clearly the effect of this chemical. All photographs were taken

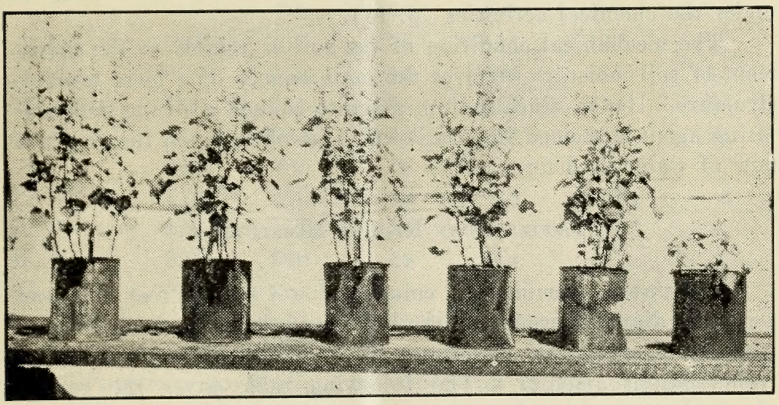

Fig. 3—Buckwheat—Soil No. 3
From left to right, pots No. 8, 1, 2,3, 4, 5.

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when the plants were about 1 month old, and some of the most stunted plants died before reaching maturity.

Arsenic determinations were made on all plants from pots numbered 4, 5 and 6. The Marsh apparatus was used in the analysis as described in U. S. Dept of Agr., Bur. of Chem., Bul. 113, p. 61. It was found to be present in all plants grown in these pots, but not in weighable amounts, working on a 5 gram sample, except pots No. 5, millet and buckwheat grown on soil No. 3. In the former case it amounted to .0018 per cent and in the latter .0053 per cent. This indicates that the arsenic passes from the soil into the plant tissues and that when present in the plant in minute quantities growth is materially stunted.

Effect upon the Physical Condition of the Soil.

One of the most noticeable effects of sodium arsenite in the pot experiments was its action upon the physical condition of the soil. It has a very strong deflocculating action which may be attributed to either one or both of two reasons. First, arsenious acid belongs to the class of weak acids such as phosphoric and others, the alkali salts of which have a strong deflocculating effect upon clay. Second, when sodium arsenite is added to the soil, a process known as selective absorption takes place, setting free the soda. This phase of the question is dealt with more thoroughly under the chemical activities (p. 13).

The mechanical condition of the soil as related to the movement of soil moisture involves the maintenance of a loose crumby structure. Hence, since sodium arsenite is such a strong deflocculating agent it seemed imperative that its effect upon the rise and flow of water be determined.

Effect upon Rise of Moisture.

For these experiments a column of soil several feet long and 1 inch in diameter was used, the bottom of the column standing in a solution containing 200 parts per million of arsenic (As_2O_3) from sodium arsenite. The following table gives the results obtained:

Time	Soil No. 1		Soil No. 2		Soil No. 3	
	Blank	Arsenic	Blank	Arsenic	Blank	Arsenic
	cm.	cm.	cm.	cm.	cm.	cm.
1 hr	16.0	15.1	15.9	16.5	22.4	21.4
3 "	20.6	19.8	22.3	22.5	34.8	33.5
7 "	24.0	23.6	28.8	27.2	46.0	44.0
22 "	29.1	28.9	37.0	34.7	60.8	58.4
46 "	33.4	33.8	44.1	38.5	69.7	67.4
68 "	35.8	36.4	49.1	41.7	75.4	72.7
72 "	37.6	38.5	52.4	44.5	79.3	76.5
116 "	39.4	40.2	57.6	49.7	86.2	83.2
164 "	41.8	42.7			90.7	87.7

These results show the rise of water to be diminished in two soils and practically unaffected in the third.

In the next table are given data showing the effect upon the flow of water through the soil. The apparatus used was very similar to that described above except that the soil column was kept under a constant head of one inch of a solution containing 200 parts per million arsenic. The amount passing through was measured at various intervals.

EFFECT OF ARSENIC ON PERCOLATION OF WATER THROUGH SOILS.

Time	Soil No. 1		Soil No. 2		Soil No. 3	
	Blank	Arsenic	Blank	Arsenic	Blank	Arsenic
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
8 hrs	286	416	78	183	134	128
24 "	695	650	90	94	225	164
32 "	365	230	35	18	119	38
48 "	910	680	67	18	236	37
56 "	400	305	28	..	..	..
72 "	770	420	40	18	37	6
80 "	420	135	..	..	..	..
98 "	620	240	44	14	..	..
112 "	950	..	..	..	..	..

Thus it may be seen that the deflocculating action of this chemical is directly related to the movement of water. It causes the soil particles to swell, more especially in the organic soils and as the concentration increases a water logged condition ensues. It also accentuates the colloidal state thus increasing the friction and thereby making the passage of water more difficult.

Biological Influences.

Arsenic belongs to the class of chemicals known as very strong poisons. All forms of plant and animal life are influenced by these chemicals, the degree of effectiveness depending on the amount of arsenic present and the degree of resistance exhibited by the plant or animal. Among the most important lower plants within the soil are the bacteria which ammonify and nitrify the nitrogenous material, thus rendering the inert componds of this element available to the plants. The rate of ammonification or nitrification is even greatly influenced by the presence in the soil of excessive amounts of salts generally considered comparatively

inert[5]. Thus their development and functions would be expected to be greatly influenced by the presence of such a virulent poison as arsenic.

Ammonification.

For determining the influence of arsenic toward the organisms which bring about ammonification the following method was used. One hundred grams of soil and 1 gram of dried blood were weighed into glass tumblers, arsenite of soda added in varying amounts, the moisture content brought up to the optimum point and the whole well stirred. They were then incubated for 7 days and the ammonia determined by the usual method. Results are given in the following table:

EFFECT OF ARSENIC ON AMMONIFICATION.

Arsenic in soil per cent.	Soil No. 1 Ammonia in 100 gms. soil mgs.	Soil No. 2 Ammonia in 100 gms. soil mg.	Soil No. 3 Ammonia in 100 gms. soil mg.
.001	35.5	98.4	40.5
.005	21.3	75.3	43.0
.010	27.8	83.3	51.5
.015	35.1	76.1	48.0
.050	28.5	71,4	70.4
.100	33.0	81.2	90.0
.250	34.0	76.0	100.1
.500	..	..	85.8
1.000	..	..	99.1
Check	34.5	87.1	53.7

From the above results it may be seen that in some proportions a stimulation takes place while in others a retarding influence is noted. No general law is applicable to all soils. The greatest stimulation is noted in the organic soil (No. 3), being at .25 per cent As_2O_3 almost double that of the check. A strong stimulation is also apparent even at the excessive content of one per cent. The least stimulation is to be found in the soil of heaviest clay content. Hence an obvious conclusion to be reached from these results is that the influence of sodium arsenite on ammonification depends primarily upon the mechanical composition of the soil

and possibly to some extent upon the presence or absence of organic matter.

Nitrification.

In order to study the influence upon the nitrifying bacteria, the same method described under the determination of the ammonifying power was used except that the incubation extended over a period of 21 days. The per cent nitrates in the soil was then determined by the usual method. The results are given in the following table.

EFFECT OF ARSENIC ON NITIFRICATION.

Arsenic in soil	Soil No. 1 Nitrogen As NO_3	Soil No. 2 Nitrogen as NO_3	Soil No. 3 Nitrogen as NO_3
percent.	p.p.m.	p.p.m.	p.p.m.
.001	82	310	455
.005	114	280	350
.010	240	275	350
.015	66	275	320
.050	400	210	330
.100	88	260	375
.250	24	260	405
Check	80	300	370

The influence of sodium arsenite on the nitrifying bacteria was greatest in the red clay soil (No. 1). Here there was a decided stimulation up to .05 per cent, followed by a rapid reduction in nitrate content. In the other soils the influence was less marked. In these the functions of the bacteria were apparently little influenced even by excessive amounts of the arsenic. In soils 1 and 3 there was little correlation in the influence upon the ammonifying and nitrifying bacteria while in soil No. 2 the action was very similar.

Chemical Activities.

When any chemical salt is added to a soil it either combines with or replaces substances already present or remains inactive and is readily washed out by the rains. Still other salts are broken up or decomposed after being added to the soil, one part being washed out while the other remains fixed. This process is known

as selective absorption. Most salts belong to the first or second class and it is of the greatest importance that we know whether arsenic remains fixed in the soil or is washed out by the rains. If it is inactive then it may be considered harmless but if active toward substances already present then a judicious use is imperative.

The first series of experiments to determine the action of arsenic upon the soil from a chemical view point, were made with the use of large glass percolators, 800 grams of soil being placed in each of six percolators. The soils used were the same three as used in the pot experiments. In this case 500 cc of a solution of sodium arsenite containing 184 parts per million arsenic was poured into the percolator every day for 3 days and the analysis of the filtrate made to determine how much arsenic had been taken out by the soil. These simple tests indicated a strong affinity of soils for arsenic, the first portions of the filtrate being absolutely free of this chemical. Later portions passing the soil contained slight traces.

In view of the above results a more thorough test was made upon these same soils with lysimeters, using about 25 pounds of soil. A solution of sodium arsenite of the same strength as used in spraying was prepared and about 3 litres (approximately 3 quarts) poured on the soil in each lysimeter. Two lysimeters were filled with each soil, to one of which the 3 litres of arsenic spray was applied and the lysimeter allowed to stand about 2 months. Samples of the soil were then taken at different depths to determine how deep the arsenic had penetrated and to what extent it had distributed itself at various depths. To the second lysimeter one quart of water was added every other day for several weeks, then allowed to remain until dry enough to sample. Samples were then taken at different depths to determine to what extent the arsenic is carried down by the movement of the water.

ARSENIC (As_2O_3) AT DIFFERENT DEPTHS IN SOIL IN LYSIMETERS.

	Soil No. 1		Soil No. 2			Soil No.3	
Depth	Not leached p. cent.	Leached p. cent.	Not leached p. cent.	Leached p. cent.	Depth	Not leached p. cent.	Leached p. cent.
1-3 in.	.280	.224	.450	.237	1-2 in.	.97	.95
3-5 in.	.198	.211	.170	.092	2-4 in.	.50	.47
5-7 in.	.171	.145	.118	.092	4-6 in.	.00	.00
7-9 in.	.184	.170	.013				

In the above table the first column represents the depths to which samples were taken from soils 1 and 2. Soil No. 3 was sampled at different depths as indicated in column 6. Apparently the organic matter is most active toward arsenic as in the highly organic soil (No. 3) it was practically impossible to wash out the arsenic. These data show the extent to which arsenic is held in the soil by absorption and indicate the danger to be expected from the accumulation of excessive applications.

The tract of land which has been sprayed longest in these islands is at Nahiku. Here the weeds have been kept down for the last 5 years by three sprayings per year at the rate of 5 pounds per acre per application. No plowing has been done and hence the soil has remained undisturbed. During this time the rainfall has amounted to about 200 inches per year, and the soil during this time has been subjected to heavy washing. A large percentage of the water has penetrated into the soil as the loss of water through surface washing is very low in this district. Through the kindness of Mr. Anderson samples were obtained from this district to a depth of 12 inches, being taken every 4 inches. The first 4 inches contained .00924 per cent arsenic (As_2O_3), the samples below this depth contained none at all. This is entirely in accord with the results obtained in the lysimeters.

The nature of the reaction between the sodium arsenite and the soil is apparently one of complex proportions. The greatest activity is with the iron and humus and to a less extent the lime and magnesia. A complete analysis of the filtrate collected from the percolation experiments, using a solution containing 200 parts per million arsenic, disclosed very little conclusive data. However, the concentration was, in most instances, found to be greatest in

the filtrates obtained by passing the solution of sodium arsenite through the soil as compared with a water extract.

In order to magnify the action of sodium arsenite a series of experiments was planned as follows: 200 grams each of the 3 types of soil was weighed into a large bottle and to this was added 1 litre of a solution containing 1 per cent sodium arsenite. Check experiments were also made upon the soils using 1 litre of water. These were well shaken at intervals for several days and allowed to stand two weeks. They were then filtered and analyses made on the filtrates, and humus determined in the soils.

The following table shows the percent of humus removed from the soil by treatment with sodium arsenite.

HUMUS REMOVED FROM THE SOILS BY TREATMENT WITH 1 PER CENT. SODIUM ARSENITE SOLUTION.

Soil Number	Humus in soil. per cent.	Humus in soil after treatment per cent.	Humus dissolved. per cent.
1	2.77	1.56	1.21
2	1.68	1.80	0.00
3	8.75	8.40	0.35

In the next table is presented data relating to the changes in composition of the sodium arsenite solution when in contact with the soil.

ANALYSES OF WATER AND ARSENIC EXTRACTS OF SOILS.

	Soil No. 1		Soil No. 2		Soil No. 3	
	Water extract mg.	Arsenic extract mg.	Water extract mg.	Arsenic extract mg.	Water extract mg.	Arsenic extract mg.
Iron oxide	trace	716.0	trace	121.0	trace	90.0
Calcium oxide ...	11.2	84.0	13.6	124.0	74.6	126.
Magnesium oxide	3.6	20.0	10.8	44.0	7.4	26.0
Arsenic (As_2O_3)..	..	396.0	..	6000	..	4480.0
Arsenic fixed by 200 gms. soil..	..	2640	..	600	..	2120.0

The above figures represent miligrams per litre, excepting the last column which gives miligrams of arsenic (As_2O_3) fixed by the soil. An analysis of the solution used shows an arsenic content of 6600 miligrams per litre.

From these data it is possible to advance several theories as to the action of this chemical. The soluble action toward humus is

undoubtedly a result of selective absorption. That is, the arsenic is absorbed and the soda liberated as the carbonate or possibly hydrate. The action of sodium carbonate toward humus is well known, it being responsible for the black alkali lands of the western states. The supposedly neutral salt of sodium arsenite is alkaline in reaction and as long as it is in solution in contact with the soil it remains alkaline, at least in so far as these experiments were carried out. But soon after filtering the soil the solution becomes acid or neutral or possibly may remain alkaline depending on the degree of absorption which took place when in contact with the soil.

As additional proof of the theory that selective absorption takes place it should be stated that the soil absorbing the highest weight of arsenic was in contact with the solution of highest alkalinity and lost, through solution, the most humus. The soil absorbing the least arsenic lost no humus.

The extract of the soil treated with arsenic was very dark in color according to the humus dissolved. On acidifying this extract with hydrochloric acid a large per cent of the humus was precipitated, carrying with it a large percentage of the iron. This is a well known property of humus as extracted from soils by ammonia.

The most striking figures are those showing the increase in solubility of iron as effected through the presence of sodium arsenite. The solubility of this constituent of our soils is very low. It is present in a form soluble in water, in only minute traces.

The interaction of the arsenic and iron apparently depends upon the form in which the latter exists in the soil. Soil No. 1 is a red clay and here the reaction is strongest, soils number 2 and 3 are a brown and a black soil respectively and the action is far less marked. What the nature of the action is it is impossible at this time to say as it would involve the establishing of the presence or absence of ferrates or ferrites in the soils. This involves an investigation too technical for a bulletin of this sort. Further work of this nature is contemplated. The reaction with the red soil is primarily chemical, the arsenic replacing the iron and the latter going into solution. It is first necessary to determine definitely the chemical form in which the iron exists in the soil in order to present a theory as to the nature of the reaction.

The preceding table shows the red soil to have absorbed the most arsenic, while the results in the lysimeters show the organic soil to have the highest absorbing power. This is due to the fact that the arsenic solution acts more slowly upon the physical properties of the red soil and hence in the lysimeters penetrates to a greater depth before fixation is complete. Further proof of this may be had by reference to the tables showing effect on capillary rise and fall of water. The movement in the red soil is influenced least by the arsenic.

Summary.

1. Arsenite of soda has been used as a spray for killing weeds in Hawaii for about five years.

2. As a means of eradicating these pests it has proved cheap and effective.

3. Two formulas may be used in preparing the spray. One results in a neutral sodium arsenite, the other in an acid sodium arsenite. The former is the more economical, adds less arsenic to the soil, and is equally efficient as a weed destroyer.

4. Plant growth is very sensitive to this poison, the resistance varying with the different plants and the different types of soil. It is assimilated by the plant and on analysis of the dead plants may be found scattered throughout the tissues.

5. The mechanical condition of the soil is materially altered by its presence. Its action is primarily that of a deflocculating agent, thus checking the movement of the water.

6. The influence upon the soil bacteria varies considerably in the different types of soil and no general rule seems to apply within reasonable limits.

7. This chemical is strongly fixed by the soil even resisting the washing of excessive rains, and is shown to accumulate in the top layer. A sample of soil taken from a tract of land sprayed for five years, on analysis showed all the arsenic to be present in the top four inches of soil.

While there is no immediate danger from the use of arsenite of soda in killing weeds it is imperative that it should not be used in excessive amounts if, as these experiments indicate, the accumulation in the top few inches of the soil is to continue indefinitely.

REFERENCES.

[1] Press Bul. 30, Haw. Exp. Sta.
[2] Colorado Exp. Sta. Buls. 131 and 157.
[3] Bur. Chem. U. S. Dept. Agri., Bul. 39.
[4] Press Bul. 30, Haw. Exp. Sta.
[5] Biochem. Bul. 3 (1913), pp. 2-16.